Performance Monitoring Protocol (QA/QC) for the Thermo LTQ LC/MS (ESI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo LTQ LC/MS system consisting of a Thermo Electron LTQ Mass Spectrometer (MS) and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/ equipment in Quantico, VA in the following disciplines/categories of testing: Drug chemistry, toxicology, explosives (chemistry), and Chemistry Unit general physical and chemical analysis.

2 Principle

The LTQ system is comprised of a Shimadzu LC and a Thermo Electron Linear Ion Trap LTQ MS. The instrument is configured with an API source that is capable of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI and APPI provided the method of ionization is clearly labeled in the resulting data and documentation. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

a. Instrumentation - Thermo Electron LTQ MS, API Source, Shimadzu Prominence LC, and Data System with XCalibur software (or equivalent)

b. API Gas - Nitrogen, 99.99% (high purity or equivalent)

c. Ion Trap Gas - Helium, 99.99% (high purity or equivalent)

d. Methanol, Optima grade or equivalent

e. Deionized Water, 18 MΩ·cm Milli-Q or equivalent

f. Acetone, HPLC grade

g. Ammonium Nitrate (NH_4NO_3), reagent grade

h. Pierce ESI Positive Ion Calibration Solution (Thermo or equivalent)

i. Caffeine (Sigma or equivalent)

j. Pierce ESI Negative Ion Calibration Solution (Thermo or equivalent)

k. Ammonium Hydroxide (NH_4OH), reagent grade

l. Codeine (Sigma or equivalent)

m. Brucine (Sigma or equivalent)

n. Reserpine (Sigma or equivalent)

o. γ-Aminobutyric Acid (GABA), (Sigma or equivalent)

p. HMX, RDX, Tetryl, NG, PETN standards at 1000 µg/mL (Cerilliant or equivalent)

q. HMTD standard at 1000 µg/mL (Cerilliant or equivalent)

r. Volumetric glassware

s. Infusion Syringe - 10 to 500 µL LC syringe (Hamilton or equivalent)

t. Basic LC Mobile Phase (95:5:0.03 Methanol:Water:Ammonium Hydroxide), or appropriate discipline specific mobile phase.

u. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)

v. C-18 Column (Grace Altima or equivalent)

4 Standards and Controls

4.1 Testmix (Toxicology/General Chemistry)

The testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. Record all preparations in the Reagent Log. To prepare:

a. Stock Solution - Weigh 1.5 mg GABA, 5.0 mg caffeine, 1.0 mg codeine, 1.0 mg brucine, and 1.0 mg reserpine into a 100-mL volumetric flask. Bring to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.

b. Testmix Solution - Pipette 4.0 mL of the Stock Solution into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. Shelf life is three years when stored refrigerated in brown glass. This preparation may be appropriately scaled.

4.2 Testmix (Explosives Chemistry)

4.2.1 Explosives by ESI

The testmix is used to assess daily operating performance, mass assignment, and continued

integrity of the system. Record stock solution preparations in the Reagent Log. To prepare:

a. Stock Solutions – place 1 mL of each 1000 µg/mL of HMX, RDX, Tetryl, NG and PETN standards in a separate 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 100 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

b. Testmix Solution - Pipette 1 ml of each 100 µg/mL stock solution of HMX, RDX, Tetryl, NG, and PETN into a 10 mL volumetric flask and dilute to the mark with acetone to achieve a final concentration of 10 µg/mL. Shelf life is two years when stored refrigerated in colored glass. This preparation may be appropriately scaled.

4.2.2 Explosives by APCI

The HMTD Testmix is a 10 µg/mL solution of HMTD in deionized water. This solution must be prepared fresh each day. To prepare the testmix, add 10 µL of the 1000 µg/mL stock solution of HMTD to an autosampler vial, then add 990 µL of 18.2 MΩ·cm deionized water.

4.3 Calibration Solution

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

5 Calibration

The calibration procedure should be performed as needed, when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

a. Load a 250 µL syringe with the appropriate calibration solution.

b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.

c. Set the syringe pump to the correct syringe type and set the pump rate to 10 µL/minute.

d. Load the tune file “esi_tune” (or equivalent).

e. Check that instrument is in POSITIVE ION mode and collecting CENTROID data.

f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of

this protocol.

g. Turn on the syringe pump and verify that the solution is flowing out the ESI needle.

h. Engage the ESI probe and turn on the MS.

i. In Tune Plus, open the Calibrate dialog box, choose the 'Automatic' tab and check the individual tests or 'Select All' and then 'Start.'

j. When the calibration is complete, it will display whether or not the calibration was successful.

- If the procedure fails, repeat the calibration.
- When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the spectrum of the calibration solution.

k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, the IOSS Manager or appropriate instrument support personnel will determine the corrective maintenance to be performed.

6 Sampling or Sample Selection

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps will be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

a. Record the remaining disk space on the hard drive. Use either the Windows Explorer or Xcalibur program verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain. If analysis consists of multiple samples in a sequence, ensure that there is additional sufficient storage space.

b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100 p.s.i. If it cannot maintain this pressure, contact appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 p.s.i. remaining.

c. Record the line pressure of the building helium supply (ion trap gas). The regulator should read between 20 and 40 p.s.i. (30 – 60 p.s.i. if two instruments are to be run

off the same regulator). If it cannot maintain this pressure, contact appropriate instrument support personnel. If the helium is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 100 p.s.i. remaining.

d. Check the Vacuum Pressure to ensure that no significant leaks are present in the system. Do not use if the Convectron Gauge reads above 2 torr, or the Ion Gauge (if present) reads above 20 microtorr.

e. If using a Shimadzu LC System, prime each LC solvent line to be used that day. Open the prime valve on the front of each pump module to be used by turning the valve handle ninety degrees and press the "PURGE" button on the module. If the pump does not start priming, disengage remote control by pressing the "PUMP" button and then pressing the "PURGE" button again. After the prime cycle finishes (about three minutes), close the prime valves.

f. If using the Shimadzu LC System, the autosampler solvent wash can be primed by pressing the "PURGE" button on the front of the autosampler module.

g. Prepare the instrument for analysis of testmix. Verify that the instrument has the correct source probe installed (ESI), the correct tune file loaded (esi_tune, exp_tune or equivalent), positive ion or negative ion mode selected, and centroid data being collected.

h. For Toxicology/General Chemistry Testmix: If a column is installed, remove it from that system and replace it with a zero-dead-volume union.

i. For Toxicology/General Chemistry Testmix: Perform an analysis of the appropriate testmix prior to the analysis of case samples. For targeted analytes, a positive control can be substituted for the testmix. Use parameters listed in the 'Instrumental Conditions' section of this protocol. Select the appropriate mobile phase. Start the HPLC pump. Engage the ESI probe and turn on the MS. Start an acquisition using a filename such as 'TMyymmdd' (or equivalent). Make three 5 µL injections of the testmix solution at least 10 seconds apart by using the manual loop injector, and then stop the data collection. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.

j. For Explosives Testmix: Conduct a performance verification of the appropriate testmix through the column. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the testmix.

k. If all requirements are within specification, prepare the documentation as outlined in the "General Instrument Maintenance Protocol." If any requirements fail, contact appropriate instrument support personnel.

7.2 As Needed Checks

a. Replace the metal needle as needed.

b. Clean or replace the heated capillary as needed.

8 Instrumental Conditions

8.1 Testmix (Toxicology/General Chemistry)

Liquid Chromatograph

Mobile Phase:	Basic LC Mobile Phase (or discipline specific Mobile Phase)
Flow Rate:	0.3 mL/min
Column:	None
Inj Volume:	5 µL
Number of Inj:	3

Mass Spectrometer

Ionization:	ESI
Polarity:	Positive
Tune File:	esi_tune
Sheath Gas Flow:	6 (arb)
Aux Gas Flow:	3 (arb)
Sweep Gas Flow:	3 (arb)
Scan Mode:	Full Scan
Scan Range:	100-650 m/z

8.2 Testmix (Explosives Chemistry)

8.2.1 Explosives by ESI

Liquid Chromatograph

Mobile Phase:	60% Methanol : 40% 3.125 mM Ammonium Nitrate
Flow Rate:	0.3 mL/min
Column:	C-18
Inj Volume:	5 µL

Mass Spectrometer

Ionization:	ESI
Polarity:	Negative
Tune File:	exp_tune
Sheath Gas Flow:	20 (arb)
Aux Gas Flow:	5 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	200-400 m/z (minimum)

8.2.2 Explosives by APCI

Liquid Chromatograph	
Mobile Phase 1:	Methanol with 1.25 mM Ammonium Nitrate
Mobile Phase 2:	DI H_2O with 1.25 mM Ammonium Nitrate
Flow Rate:	0.3 mL/min
Gradient:	0-2 min 90% Mobile Phase 2
	12-14 min 50% Mobile Phase 2
	17-20 min 90% Mobile Phase 2
Column:	C-18
Inj Volume:	10 µL
Mass Spectrometer	
Ionization:	APCI
Polarity:	Positive
Tune File:	HMTD_TUNE (or equivalent)
Sheath Gas Flow:	35 (arb)
Aux Gas Flow:	15 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	150-250 m/z (minimum)

8.3 Calibration

Mass Spectrometer	
Ionization:	ESI
Tune File:	esi_tune
Scan Mode:	Full Scan
Scan Range:	100-2000 m/z

9 Decision Criteria

9.1 Testmix (Toxicology/General Chemistry)

Verify the results of the testmix. The following ions should be observed in the three testmix injections: RICs should show contemporaneous signals for components at the following masses:

- Caffeine 195 m/z
- Codeine 300 m/z
- Brucine 395 m/z
- Reserpine 609 m/z

9.2 Testmix (Explosives Chemistry)

9.2.1 Explosives by ESI

Verify the results of the testmix. RICs should show contemporaneous signals for components at the following masses:

- HMX ($+NO_3$) 358 m/z
- RDX ($+NO_3$) 284 m/z
- Tetryl ($+NO_3$) 349 m/z
- NG ($+NO_3$) 289 m/z
- PETN ($+NO_3$) 378 m/z

9.2.2 Explosives by APCI

Verify the results of the testmix. RICs should show contemporaneous signals for components at the following masses:

- HMTD 224, 209, 207 m/z

9.3 Calibration

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

- Caffeine 195 m/z
- MRFA 524 m/z
- Ultramark 1022 m/z
  1122 m/z
  1222 m/z
  1322 m/z
  1422 m/z
  1522 m/z
  1622 m/z
  1722 m/z
  1822 m/z
  1922 m/z

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (Inst 001) *Instrument Operation and Systems Support SOP Manual.*

"Liquid Chromatograph General Maintenance Protocol" (Inst 003) *Instrument Operation and Systems Support SOP Manual.*

"Mass Spectrometer General Maintenance Protocol" (Inst 004) *Instrument Operation and Systems Support SOP Manual.*

"Preparation of Chemical Reagents" (Tox 103) *Toxicology SOP Manual.*

“Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-Tandem MS” (Tox 418) *Toxicology SOP Manual.*

FBI Laboratory Safety Manual.

Rev #	Issue Date	History
2	04/25/16	Updated section 3 to include standards for Explosive Chemistry, calibration solutions, as well as mobile phase and column selection. Removed Ultramark 1621, MFRA, acetonitrile, ammonium formate, formic acid, and acetic acid. Explosives Chemistry Testmix added to section 4.2. Removed preparation of calibration solution in section 4.3. Differentiation made between testmix procedure for Toxicology/General Chemistry and Explosives Chemistry, section7.1. Section 7.2 removed capillary tubing. Explosives Chemistry Instrument conditions placed in section 8.2. Section 9.2 updated for Explosives Chemistry Decision Criteria.
3	04/29/16	Section 4.2 fixed typo and added that preparations may be appropriately scaled. Changed wording in section 7.1 to allow Windows Explorer to be used to check disk space. Section 8.2 corrected for Mobile Phase concentrations.
4	10/04/18	Updated Section 1 Scope to include applicable disciplines/categories of testing. Changed Section 3 t to 250 mg. Added HMTD to Section 3 q. Changed ‘all’ to ‘stock solution’ in Section 4.2. Added ‘Explosives by ESI’ for clarification in Sections 4.2.1, 8.2.1, and 9.2.1. Added Sections 4.2.2, 8.2.2, and 9.2.2 for ‘Explosives by APCI’ and HMTD Testmix. Updated heading in Section 6. Added ‘appropriate instrument support personnel’ to Sections 5 k and 7.1 b, c, & k. Added targeted analytes to Section 7.1 j. Updated ‘Instrument Operation and Systems Support’ in Section 14 and header. Added ‘(minimum)’ to scan range in 8.2.

Approval

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Drug Chemistry/
General Chemistry
Technical Leader: Date: 09/28/2018

Toxicology
Technical Leader: Date: 09/28/2018

Explosives (Chemistry)
Technical Leader: Date: 09/28/2018

IOSS Manager: Date: 09/28/2018

Chemistry Unit Chief: Date: 09/28/2018

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QA Approval

Quality Manager: Date: 09/28/2018